



Aerobic Bacteria Isolated from Infertile Dairy Cows (Repeat Breeders) in Gezira State, Sudan

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Abstract

The aim of this research was to study the role of the aerobic bacteria persisted in vaginas of repeat breeder dairy cows in Gezira State-Sudan. One hundred and ninety two samples of sera, vaginal swabs and cervico-vaginal washes were collected from local and cross breeds of repeat breeder cows during the period May 2012 to May 2013. Serum samples were tested for brucella antibodies by Rose Bengal Test (RBT), while vaginal swabs and cervico-vaginal washes were used for bacterial isolations. The vaginal swabs were cultured on both serum dextrose agar and blood agar media for the isolation of *Brucella* spp. and other related bacteria respectively while cervico-vaginal washes were cultured on Skirrow' s selective medium for *Campylobacter*. The isolated bacteria (except *Bacillus* spp.) were identified using the VITEK[®] 2 Compact system while *Bacillus* spp. were identified according to Barrow and Feltham (2003) method. 25 %(11) of the tested sera were found positive for brucella antibodies; however, on cultures only 2 brucella isolates were recovered. On Blood Agar medium, 89.1% (57) of the vaginal swab samples showed bacterial growth and 87 bacterial isolates were recovered from these cultures. The predominant bacteria were *Staphylococcus* spp. (24.1%) followed by *Kocuria* spp. (18.4%) and both of *Granulicatella* spp. and *Bacillus* spp. (5.7%). Some bacterial spp. were isolated for the first time in Sudan and many of them such as: *Streptococcus pneumoniae*, *Streptococcus pluranimalium*, *Gardnella vaginalis*, *Providencia rettgeri*, *Streptococcus dysgalactiae* subspp. *equisimilis*, *Aeromonas hydrophila* and *Burkholderia cepacia* act as significant pathogens for bovine and human .Although the prevalence of some bacteria *Myroides* spp., *Cronobacter sakazakii* group, and *Gemella bergeri* in animals was not globally proven yet, they were found in this study inhabiting the vaginas of repeat breeder cows which is an unexpected findings. *Campylobacter fetus* bacterium was not isolated from the cervico-vaginal washes.

Keyword: Brucella app, Staphylococcus app, Kacuria app, Repeat breeder.

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Introduction

Repeat breeding (RB) is a situation when the cows require three or more services to conceive and inter-calving interval thereby is prolonged causing low milk and low calf

production resulting in greater economic losses to dairy industries (Enkhia *et al.*, 1983, Singh *et al.*, 2008). A cow reported as a repeat breeder when aged less than 10 years old, has calved before with normal healthy

cycle, no palpable abnormalities and then bred three or more times without getting conceived. Pregnancies of RB cows always lost before 42 days of gestation (Chakurkar *et al.*, 2008, Hovingh, 2009). In studies done to determine the fertilization rate of a cow herd showed clearly that early embryonic death is the main cause of cow repeating following service. The great majority of embryonic deaths occurring between 3 and 34 days ; actually occur between the end of 16 days of gestation and the day the cow returns to heat. A number of factors can cause RB, including reproductive tract infection, sub-fertile bulls, endocrine problems, malnutrition and poor management (Mukasa-Mugerw, 1989). After parturition, it is more favorable to pathogenic invaders such as *Campylobacter* spp., *Brucella* spp., *Leptospira* spp. and *Trichomonas* spp. and also to opportunistic microorganisms to cause infections. Generally, non specific infection of the genitalia is considered to be the main cause of repeated conception failure (Singh *et al.*, 1996). Bacterial infection may cause cervicitis or endometritis of various degrees which in turn lead to embryonic deaths and RB problems (Elliott *et al.*, 1968). The predominant organisms isolated from uterus of RB cows were: *Micrococcus luteus*, *Staph. aureus*, *Staph. epidermidis* and *E.coli*, while *Aerococcus viridians*, *Klebsiella* spp., *Staph. aureus* and *Micrococcus luteus* were found to be predominant in the cervix of RB cows (Salwa, 1983), however, Gani *et al.*, (2008) found the main predominant bacteria in the uterus of the repeat breeders were: *Staphylococcus* spp. followed by *Bacillus* spp., *E.coli*, *Pseudomonas* spp., and then other Gram negative rod bacteria. Alsayed (1994) isolated *A. pyogenes*, *Staph. aureus*, *Staph. epidermidis*, *E. coli*, *Ps. mallei* and *Str. fecalis*.

Gezira State is a quite source of house hold income and contributed in production of food, milk and export in Sudan. The problem

of infertility as general and RB in particular in cattle is a serious problem that faces the producers and veterinarian in Gezira State. The identification of the infectious agents is really important because appropriate treatment or control measures should be specific and needs to be adjusted for each individual situation. This study was conducted in Gezira State- Sudan and aimed to isolate and identify the bacteria encountered in the RB problem and subsequently to determine the predominant bacteria colonizing the vaginas of RB cows using a newly introduced and more reliable technique for identification, VITEK[®] 2 Compact system.

Materials and Methods

Sampling

During the period of May 2012 to May 2013 a total of 192 samples including: sera (n=64), vaginal swabs (n=64) and cervico-vaginal washes (n=64) were collected from 4 to10 years old cross cow breeds, (cross Friesian and cross Jersey, n=45) and local breeds, (Kenana and Butana, n=18) with history of RB (data of one breed was missed). RB was diagnosed according to Chakurkar *et al.*, (2008). The collected samples were from different localities in Gezira State, and were used for serological and bacteriological examinations.

Serological tests

The blood sera were separated and tested for brucella antibodies using Rose Bengal test.

Bacterial isolations and Identification

Vaginal swabs collected which their sera showed positive results for brucella antibodies were cultured on serum dextrose agar medium, incubated at 37°C under 5-10% carbon dioxide in a candle jar for up to 5 days of incubation (Alton *et al.*, 1975). For isolation of other related bacteria, all vaginal swabs were cultured on Blood Agar medium, incubated aerobically at 37°C for 24 hrs. The

cervico-vaginal washes were cultured (according to OIE Manual, 2008) on Skirrow's selective medium for *Campylobacter*, incubated at 37°C under microaerobic conditions obtained by gas generator kits (Campy Gen, Oxoid). Cultures which showed bacterial growth were then sub-cultured before they further used for bacterial identification. With the exception of *Bacillus* spp. isolates, all other isolates were identified using the automated system VITEK[®] 2 Compact (BioMérieux[®], 21CFR Part 11 compliant software). The manufacturer's instructions were followed for the inocula suspension and incubation of isolates (BioMérieux, 2004). *Bacillus* spp. isolates were identified according to Barrow and Feltham (2003).

Results

Sixteen (25%) of the tested blood sera samples were positive for brucella antibodies by means of Rose Bengal tests (RBT), while 48(75%) were negatives. Only 2 isolates of *Brucella* spp. were recovered from the vaginal swabs samples cultured on serum dextrose agar medium. 57(89.1%) of the vaginal swabs which were cultured on blood agar medium, showed bacterial growth and a total of 43 bacterial spp. (87 isolates) were recovered from these cultures. The Gram positive bacterial spp. were 29 while the Gram negatives were 14. *Campylobacter fetus* bacterium was not isolated on the Skirrow's selective medium for *Campylobacter*.

The Gram positive and Gram negative bacterial spp. isolated from local and cross breeds of repeat breeder cows in all Gezira State localities were shown in Table (1 and 2). The predominant bacteria found in RB cow vaginas were *Staphylococcus* spp. (24.1%) followed by *Kocuria* spp. (18.4%) and both of *Granulicatella* spp. and *Bacillus* spp. (5.7%). Some of these isolates were reported for the first time in Sudan from

repeat breeder cows such as: *Streptococcus pluranimalium*, *Streptococcus thoralensis*, *Streptococcus pneumoniae*, *Streptococcus dyssagalactiae* subspp. *equisimilis*, *Gemella bergeri*, *Gardenella vaginalis*, *Demacoccus nishinomiyaensis*, *Granulicatella* spp., *Kocuria* spp., *Aeromonas hydrophila*, *Myroides* spp., *Sphingomonas paucimobilis*, *Burkholderia cepacia*, *Providencia rettgeri*, *Acinetobacter lwoffii*, *Neisseria cineria* and *Cronobacter sakazakii* group.

In addition to the previously mentioned bacteria, some other known pathogens were isolated from cow reproductive tracts such as: *Brucella* spp., *Staph. aureus*, *E. coli*, *Staph. haemolyticus* and *Staph. epidermidis*.

Discussion

The aerobic bacteria associated with repeat breeder (RB) cows in Gezira State were studied. Serological tests and bacterial isolations and identification were done to determine the predominant bacteria inhabited the vaginas of RB cows. *Brucella* seroprevalence in this study was 25 %; the high percentage of positive cases of detected antibodies was expected due to the fact that Rose Bengal test used in this study is highly sensitive but nonspecific and therefore false positive reactors are frequently encountered (Ahmed *et al.*, 2011) which was noticed in this research, sensitivity of the test was 100% while specificity was 77%. These percentages are higher than the findings of Abdallah and Hamid, (2012) who found that 19.7% of brucella suspected cows were Rose Bengal positive while the recovery of *Brucella* spp. on brucella medium was 7.5%. In this work the recovery from brucella medium was 12.5%. *Campylobacter fetus* subspp. *veneralis* was not reported in Sudan, according OIE (2009"last accessed 2013"). In the present study, *C. fetus* was not isolated from the cervico vaginal washes taken from 6 4cows with history of RB. In this study the

vaginal swab cultured on Blood Agar medium showed that 89.1% of samples were positive for bacterial growth while in a study done by Nanda (1995), she found the positive ones were 95%. Taubrich (1958) observed that about 20% of the vaginal swabs of healthy cows were negative for bacterial growth in culture media while about 65% were inhabited by streptococci, 40% micrococci, 14% coliforms, 7% diphtheroids and 2% anthracoides organisms. In this research, the vaginal bacterial flora of the infertile cows were dominated by *Staphylococcus* spp. (24.1%), *Kocuria* spp. (18.4%) and both of *Granulicatella* spp. and *Bacillus* spp. (5.7%) while Salwa, (1983) showed that the predominant organisms isolated from uterus of RB cows were: *Micrococcus luteus*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *E.coli*, while *Aerococcus viridians*, *Klebsiella* spp., *Staphylococcus aureus* and *Micrococcus luteus* were found to be predominant in the cervix of repeat breeders. Bacteria isolated of significance from cervical mucus included *Actinomyces pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and hemolytic *E.coli*, these were incriminated as potential pathogens which render female genital tract harmful to viability of the sperms (Gunter *et al.*, 1955). This is related to our findings that *E. coli* and *Staphylococcus aureus* which have been isolated from RB cows are more likely to be responsible for RB cases. *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Streptococcus pneumoniae*, *Corynebacterium* spp., *Enterobacter aerogenes*, *Klebsiella oxytoca* and *Citrobacter diversus* were isolated from vaginal discharges of cows (Abd El-Kader

and Shehata, 2001). In this study, 3 isolates of *Staphylococcus epidermidis* were detected in repeat breeder cows, only one of them showed purulent vaginal discharges and history of abortion in addition to repeat breeding, however, *Streptococcus pneumoniae* was isolated from a repeat breeder cow, showing normal vaginal mucous. Gani *et al.*, (2008) detected *Pseudomonas* spp. and Gram negative rod minute bacterium in RB cows with mucopurulent vaginal discharges, while *Pseudomonas* spp. was isolated in this research from RB cows with normal vaginal mucous. In this study, some bacterial species were isolated for the first time in Sudan and some of them were not detected before in RB cows such as: *Streptococcus pneumoniae*, *Streptococcus pluranimalium*, *Gardnerella vaginalis*, *Providencia rettgeri*, *Streptococcus dysgalactiae* subspp. *equisimilis*, *Aeromonas hydrophila* and *Burkholderia cepacia* which considered of significance in animals and human infections.

Conclusion and Recommendation

The isolation of human- specific pathogens which were not isolated before from cows or cow's vaginas highlighted the hypothesis that human or human environment have a role in transmitting bacteria to animals which re-transmit them to human in a reverse direction. This reverse transmission poses serious threat to human health, especially to veterinarians and animal attendance. Many bacterial isolates which were isolated for the first time in Sudan from repeat breeder cows; their role in animal pathology should be explained.

Table 1: Gram positive bacteria isolated from repeat breeder dairy cows in Gezira State.

Bacterial spp. isolated	Total No.	Breed				
		CF	CJ	K	B	K × B
1- <i>Staphylococcus aureus</i>	1	1				
2- <i>Staphylococcus epidermidis</i>	3	2*				
3- <i>Staphylococcus hominis</i>	3	3				
4- <i>Staphylococcus chromogenes</i>	7	6	1			
5- <i>Staphylococcus scuri</i>	2		2			
6- <i>Staphylococcus arlettae</i>	1		1			
7- <i>Staphylococcus simulans</i>	2	1	1			
8- <i>Staphylococcus vitulinus</i>	1	1				
9- <i>Staphylococcus haemolyticus</i>	1	1				
10 – <i>Streptococcus throlatensis</i>	1	1				
11- <i>Streptococcus pneumoniae</i>	1	1				
12- <i>Streptococcus dysagalactiae</i> subsp. <i>equisimilis</i>	1			1		
13- <i>Streptococcus pluranimalium</i>	1				1	
14- <i>Kocuria varians</i>	2	2				
15- <i>Kocuria rosea</i>	9	6	1		2	
16- <i>Kocuria kristinae</i>	5	1	1		3	
17- <i>Enterococcus raffinosus</i>	1		1			
18- <i>Enterococcus facium</i>	1	1				
19- <i>Aerococcus viridans</i>	2	1	1			
20- <i>Gemella bergeri</i>	1	1				
21- <i>Gardenella vaginalis</i>	1	1				
22- <i>Dermacoccus nishinomiyaensis</i>	2	1	1			
23- <i>Micrococcus luteus</i>	1	1				
24- <i>Bacillus mucoides</i>	1	1				
25- <i>Bacillus sphaericus</i>	1	1				
26- <i>Bacillus thuringiensis</i>	2	1			1	
27- <i>Bacillus pantothenics</i>	1				1	
28- <i>Granulicatella adiacens</i>	3	3				
29- <i>Granulicatella elegans</i>	2				1	1
Total	60	38	10	1	9	1

CF= Cross Friesian, CJ= Cross Jersey, K= Kenana, B= Butana, K×B= Kenana cross Butana. *=One breed data missed.

Table 2: Gram negative bacteria isolated from repeat breeder dairy cows in Gezira State.

Bacterial spp. isolated	Total No.	Breed				
		CF	CJ	K	B	K×B
1- <i>Sphingomonas paucimobilis</i>	3	2	1			
2- <i>E. coli</i>	3	2	1			
3- <i>Pseudomonas fluorescense</i>	1	1				
4- <i>Pseudomonas pseudoalcaligenes</i>	1	1				
5- <i>Pseudomonas stutzeri</i>	1			1		
6- <i>Rhizobium radiobacter</i>	4	2	1		1	
7- <i>Burkholderia cepacia</i>	2	2				
8- <i>Aeromonas hydrophila</i>	2	1			1	
9- <i>Acinetobacter lwoffii</i>	2	1		1		
10- <i>Providencia rettgeri</i>	1	1				
11- <i>Neisseria cineria</i>	1	1				
12- <i>Myroides</i> spp.	2	1		1		
13- <i>Cronobacter sakazakii</i>	1	1				
14- <i>Alcaligenes faecalis</i> subsp. <i>faecalis</i>	3	3				
Total	27	19	3	3	2	0

CF= Cross Friesian

CJ= Cross Jersey

K= Kenana

B= Butana

K×B= Kenana cross Butana

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البكتريا الهوائية المعزولة من أبقار الألبان قليلة الخصوبة (الأبقار متكررة التلقيح) بولاية الجزيرة، السودان

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المستخلص

الغرض من هذا البحث هو دراسة دور البكتريا الهوائية الموجودة في مهابل الأبقار قليلة الخصوبة في ولاية الجزيرة بالسودان . جمعت 192 عينة من أمصال الدم، المسحات المهبلية و كذلك غسولات عنق الرحم والمهبل من أبقار تعاني من تكرار التلقيح ذات سلالات محلية وهجين خلال الفترة من مايو 2012 وحتى مايو 2013. تم اختبار عينات أمصال الدم للتحقق من وجود مستضدات البروسيليا بواسطة اختبار الـ روز بنغال، بينما استخدمت عينات المسحات المهبلية وعينات غسولات عنق الرحم والمهبل في عزل البكتريا. زرعت المسحات المهبلية في مستنبتى آجار المصل والدكستروز وآجار الدم بغرض عزل أنواع البروسيليا والبكتريا الأخرى ذات العلاقة، على التوالي، بينما زرعت مسحات عنق الرحم والمهبل في مستنبت الأسكرو الانتقائي لعزل الكامبيلوباكتري. تم التعرف على البكتريا المعزولة (عدا أنواع العصيات) من المسحات المهبلية باستخدام جهاز الفايتهك المدمج (VITEK[®] 2 Compact system) بينما تم التعرف على أنواع العصيات باستخدام طريقة Barrow and Feltham (2003) . 25% (11) من أمصال الدم المختبرة وجدت موجبة لمستضدات البروسيليا بالرغم من عزل عزلتين فقط من البروسيليا في المزارع البكتيرية. في مستنبت آجار الدم، أظهرت 89.1% (57) من عينات مسحات المهبل نمو بكتيريا حيث تم عزل 87 عذلة بكتيرية من هذه المزارع. البكتريا الأكثر شيوعا هي أنواع المكورات العنقودية (24.1%) ، تليها أنواع الكيوكوريا (18.4%) ثم كل من أنواع القرانوبوليكاتيليا والعصيات (5.7%). بعض أنواع البكتريا تم عزلها للمرة الأولى في السودان و كثير منها مثل: السبقيات الرئوية، السبقيات البلورية، القاردينيا المهبلية، البروفيدنسيا ريتاقرى، السبقيات الخيلية والبرخولديا سيباكييا تلعب دورا هاما كباكتيريا ممرضة للأبقار والانسان. بالرغم من أن وجود بعض هذه البكتيريا مثل أنواع المايروبيدز، مجموعة الكورونوباكتري ساكازاكي و الجيميليا بيرقيري في الحيوانات لم يثبت عالميا بعد، لكنها وجدت في هذه الدراسة في مهابل الأبقار متكررة التلقيح مما يعد اكتشافا غير متوقع. لم يتم عزل باكتيريا الكامبيلوباكتري الجينية من مستنبت الأسكرو الانتقائي.